Which rhizobia nodulate which legumes in New Zealand soils?

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Abstract
Recent work which genotypically characterised rhizobia of native, crop and weed legumes in New Zealand and examined their cross-nodulation ability is reviewed and related to earlier work with focus on New Zealand pasture systems. The New Zealand native legumes were exclusively effectively nodulated by novel strains of *Mesorhizobium* which did not nodulate crop or weed legumes. Clovers, lucerne, *Lotus* and grain legumes were effectively nodulated by different genera, species and biovars of rhizobia primarily originating from inoculum. Rhizobial symbionts of white clover have established over wide areas in New Zealand. Weed legumes are effectively nodulated by different genera/species of rhizobia depending on species. Bradyrhizobia that cross-nodulate lupins, gorse, European broom and tagasaste are widespread in New Zealand.

Keywords: *Bradyrhizobium, Burkholderia, Ensifer, Mesorhizobium, Rhizobium*

Introduction
Most legume species can obtain a substantial amount of their nitrogen (N) requirements for growth from symbiotic bacteria (general term rhizobia) in root nodules and this can give them an advantage in low N soils if other factors are favourable for growth (Andrews *et al.* 2011, 2013). There are only four genera of native legumes on the main New Zealand islands. These are *Carmichaelia* (New Zealand broom), *Clianthus* (kakabeak), *Montigena* (scree pea) and *Sophora* (kowhai) (Wagstaff *et al.* 1999; Heenan 2000; Heenan *et al.* 2001). However, over the past 150 years, several legumes have become important crop plants in New Zealand agricultural systems with clovers (*Trifolium* spp.), lucerne (*Medicago sativa*) and *Lotus* spp. of greatest importance in pasture systems. Also, over 100 legume species from different continents have become naturalised in New Zealand and gorse (*Ulex europaeus*), in particular, is a major weed in high country systems (Popay *et al.* 2010).

A wide range of bacteria have been shown to form N_2 fixing nodules on legumes (Sprent 2009). Some legumes are highly specific in their rhizobial symbionts while others are nodulated by diverse rhizobia. Also, some rhizobia can form "ineffective" nodules on some legumes; these nodules do not fix atmospheric N_2. Rhizobial symbionts of the common legume crops are not native to New Zealand and specific rhizobial inoculants have been used extensively in New Zealand to produce high N_2 fixing crop plants (Greenwood & Pankhurst 1976). In the 1960s and 1970s, considerable work was carried out characterising the rhizobia associated with a range of legumes occurring in New Zealand. This work primarily classified rhizobia in relation to their growth rate on artificial medium and their ability to form nodules on different groups of legumes (Greenwood & Pankhurst 1976). However, rhizobia can now be precisely distinguished and characterised on the basis of their DNA (genotypic characterisation; Willems 2006). Here we review recent work which genotypically characterised rhizobia of native, crop and weed legumes in New Zealand and examined their cross-nodulation ability. Findings are related to earlier work with a focus on New Zealand pasture systems.

Sources of data
The literature was reviewed and all work which characterised rhizobial isolates from legumes in New Zealand on the basis of their gene sequences was considered. In addition, rhizobial strains currently recommended for use on crop plants in New Zealand by the International Collection of Microorganisms from Plants (ICMP), Landcare Research, Auckland, New Zealand are summarised. Findings are considered in relation to earlier work which characterised rhizobia primarily on the basis of their growth rate and host legume(s).

Rhizobia in New Zealand soils
The New Zealand native legumes were exclusively effectively nodulated by novel strains of *Mesorhizobium* (Weir *et al.* 2004; Tan *et al.* 2012, 2013, 2015). The New Zealand brooms and kowhai were nodulated by two separate groups of *Mesorhizobium* strains. However, all strains which nodulated New Zealand brooms and almost all strains which nodulated kowhai, could nodulate kakabeak (Weir 2006; Tan 2015). Crow *et al.* (1981) reported that rhizobial symbionts of native brooms did not nodulate a range of clovers. Recent work has shown that selected strains from New Zealand brooms and kowhai did not nodulate white clover (*T. repens*), red clover (*T. pratense)*, subterranean clover (*T. subterraneum*), lucerne, *Lotus pedunculatus,* field pea (*Pisum sativum*), field bean (*Vicia faba*), *Lupinus*

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polyphyllus, blue lupin (*L. angustifolius*), white lupin (*L. albus*), tree lupin (*L. arboreus*), gorse or any of the weed species listed in Table 2 except for *Robinia pseudoacacia* (Liu 2014; Andrews M. & Tan H.W. unpublished data). All strains tested produced non-functional nodules on *Robinia pseudoacacia* (Liu 2014).

Rhizobium leguminosarum bv. trifolii is the ICMP recommended inoculum for white clover, red clover, subterranean clover, alsike clover (*T. hybridum*) and Caucasian clover (*T. ambiguum*), although subterranean clover and Caucasian clover each need specific strains different from those for the other clovers (Table 1; Greenwood & Pankhurst 1976; Landcare Research 2015). Also, there are different recommended strains for diploid, tetraploid and hexaploid Caucasian clover (Patrick *et al.* 1994; Landcare Research 2015). Strains of *R. leguminosarum* bv. trifolii for white clover have become established over wide areas in New Zealand to the extent that in these areas there is now likely to be little or no advantage in using inoculum on white clover crops (Lowther & Kerr 2011). Some strains of *R. leguminosarum* bv. trifolii can produce non-functional nodules on kowhai and New Zealand brooms (Crow *et al.* 1981; Weir 2006; Tan 2015). Indeed, these strains are commonly isolated from native legumes growing under natural conditions emphasising how widespread these strains are. *Rhizobium leguminosarum* bv. viciae is the ICMP recommended inoculum for field pea (*Pisum sativum*), field bean (*Vicia faba*), lentil (*Lens culinaris*) and vetch (*Vicia sativa*) and it is likely that in areas where this inoculum has been used extensively, there will be no effect from further additions. This is the case for field pea on the Lincoln University farm (Nguyen 2012). Some strains of *R. leguminosarum* bv. viciae can produce functional nodules on white clover (Weir 2006) but it is not known how effective these are in promoting growth. *Rhizobium leguminosarum* bv. viciae has also been found to nodulate the weed vetches *Vicia hirsuta* and *V. disperma* in New Zealand soils (Liu 2014).

Ensifer meliloti is the ICMP recommended inoculum for lucerne and it is required when sowing lucerne into soil with no history of this crop (Black & Moot 2013). Also, it has been reported in earlier work that lucerne rhizobial strains are less persistent than clover strains in acid soils so they may have difficulty establishing in some New Zealand soils (Greenwood & Pankhurst 1976). *Ensifer meliloti* produces high rates of N_2 fixation on lucerne. However, lucerne can also be nodulated by different strains of *Rhizobium* in New Zealand soils (Wigley *et al.* 2015). Under controlled conditions, growth of lucerne was substantially greater with *Ensifer meliloti* than with these *Rhizobium* strains (Liu 2014). This indicates that the effectiveness of *Ensifer meliloti* inoculum could be reduced in soils with high populations of these *Rhizobium* strains. It is not known how widespread these *Rhizobium* strains are. *Ensifer meliloti* also nodulates King Island melilot (*Melilotus indicus*) in New Zealand (Liu 2014).

Table 1 Rhizobial genera, species, biovars and strains shown to nodulate legume crop plants in New Zealand soils

Legume	Rhizobia	Reference
Clovers (*Trifolium* spp.)	*Rhizobium leguminosarum* bv. trifolii	ICMP recommended inoculum; Tan 2015
Lucerne (*Medicago sativa*)	*Ensifer meliloti* *Rhizobium*	ICMP recommended inoculum; Liu 2014; Wigley *et al.* 2015
Lotus pedunculatus	*Bradyrhizobium* ICMP 5798, *Bradyrhizobium* ICMP 5942 *Bradyrhizobium*	ICMP recommended inocula Liu 2014
Lotus corniculatus	*Mesorhizobium loti*	ICMP recommended inoculum
Lupins (*Lupinus* spp.)	*Bradyrhizobium* ICMP 8377 *Bradyrhizobium*	ICMP recommended inoculum BASF recommended inoculum
Lupinus polyphyllus	*Bradyrhizobium*	Ryan-Salter *et al.* 2014
Field pea (*Pisum sativum*)	*Rhizobium leguminosarum* bv. viciae	ICMP recommended inoculum
Soybean (*Glycine max*)	*Bradyrhizobium japonicum*	ICMP recommended inoculum

Bradyrhizobium strains ICMP 5798 and ICMP 5942 are the recommended rhizobial inoculants for diploid and tetraploid *Lotus pedunculatus* including cv. Maku while *Mesorhizobium loti* is the ICMP recommended inoculum for *Lotus corniculatus* and serradella (*Ornithopus sativus*). *Mesorhizobium loti* is also recommended for "crop lupins" although all rhizobial isolates from lupins growing in New Zealand were found to be strains of *Bradyrhizobium* (Tables 1, 2; Ryan-Salter *et al.* 2014; Liu 2014) and the effectiveness of *M. loti* on lupins needs testing. *Bradyrhizobium* ICMP 8377 is also a recommended inoculum for serradella and crop lupins. The 'Group G' commercial inoculant recommended for annual lupins in New Zealand by BASF, Auckland, New Zealand, is a *Bradyrhizobium* that effectively nodulates *Lupinus polyphyllus* (Liu 2014). However, bradyrhizobia that nodulate *L. polyphyllus* are present across a wide range of sites in the South Island including the agricultural stand of *L. polyphyllus* at Sawdon Station, Lake Tekapo, which was established using uninoculated seed (Ryan-Salter *et al.* 2014). This is in agreement with Scott (1989) who stated that *L. polyphyllus* will nodulate in high country soil without inoculum although it was also indicated that use of inoculum could be beneficial.

A diverse range of rhizobia genera nodulated weed legumes in New Zealand soils depending on plant species: these rhizobia differed from those of New Zealand native legumes and of the major crops (Tables 1, 2). Selected rhizobial strains from all weed species listed in Table 2 did not nodulate *Carmichaelia australis*, *Clianthus puniceus* or *Sophora microphylla* (Weir 2006; Liu 2014). All weed legumes were nodulated by rhizobia in a single genus except the South African native *Dipogon lignosus* which was nodulated by *Bradyrhizobium*, *Rhizobium* and *Burkholderia* strains (Liu *et al.* 2014). Sequence data indicated that the *Bradyrhizobium* and *Rhizobium* strains were respectively *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* and it is likely that both strains were derived from crop inoculum (Table 1; Liu *et al.* 2014). Gene sequences for the *Burkholderia* strains indicated that they originated in South Africa and were somehow transported with *D. lignosus* from their native habitat into New Zealand (Liu *et al.* 2014). This was the first report of a *Burkholderia*-rhizobia in New Zealand soils.

Goats rue (*Galega officinalis*) and sulla (*Hedysarum coronarium*) are highly specific in their rhizobial symbionts and were nodulated by *R. galegae* and *R. sullae* respectively as has been reported previously for these plants sampled outside New Zealand (Liu *et al.* 2012). Sulla has been considered as a potential forage legume in New Zealand as it provides high quality, non-bloating feed (Krishna *et al.* 1990; Minneé *et al.* 2004). This plant would need a highly specific rhizobial inoculant in New Zealand. *Robinia pseudoacacia* and dally pine (*Psoralea pinnata*) were nodulated by *Mesorhizobium* strains which were different from the

Table 2 Rhizobial genera and species shown to nodulate weed legumes in New Zealand soils

Legume	Rhizobia	Reference
Dipogon lignosus	*Bradyrhizobium japonicum* *Rhizobium leguminosarum* *Burkholderia*	Liu *et al.* 2014
Goats rue (*Galega officinalis*)	*Rhizobium galegae*	Liu *et al.* 2012
Sulla (*Hedysarum coronarium*)	*Rhizobium sullae*	Liu *et al.* 2012
Robinia pseudoacacia	*Mesorhizobium*	Liu 2014
Dally pine (*Psoralea pinnata*)	*Mesorhizobium*	Liu 2014
Wild serradella (*Ornithopus pinnatus*)	*Bradyrhizobium*	Liu (2014)
Wattles (*Acacia* spp.)	*Bradyrhizobium*	Weir 2006; Liu 2014
Tree lupin (*Lupinus arboreus*)	*Bradyrhizobium*	Liu 2014; Black *et al.* 2015;
Gorse (*Ulex europaeus*)	*Bradyrhizobium*	Weir 2006; Liu 2014; Black *et al.* 2015
European broom (*Cytisus scoparius*)	*Bradyrhizobium*	Weir 2006; Liu 2014; Black *et al.* 2015
Tagasaste (*Chamaecytisus palmensis*)	*Bradyrhizobium*	Liu 2014; Black *et al.* 2015

Mesorhizobium strains of New Zealand native legumes.

Bradyrhizobium nodulated wild serradella (*Ornithopus pinnatus*), *Acacia* spp. (*A. dealbata, A. longifolia*), tree lupin (*L. arboreus*), gorse, European broom and tagasaste (*Chamaecytisus palmensis*). This is in agreement with earlier reports that serradella, lupin, gorse and European broom are normally nodulated by slow growing rhizobial strains in New Zealand (Greenwood & Pankhurst 1976). Wild seradella and *Acacia* only formed effective nodules with specific strains but tree lupin, gorse, European broom and tagasaste formed effective nodules with *Bradyrhizobium* strains from all legume species (Liu 2014). *Bradyrhizobium* that cross-nodulate *Lupinus polyphyllus*, tree lupin, gorse, European broom and tagasaste are widespread in New Zealand (Weir *et al.* 2004; Weir 2006; Liu 2014; Ryan-Salter *et al.* 2014; Black *et al.* 2015). A group of these rhizobia have some unique gene sequences and may be of New Zealand origin (Ryan-Salter *et al.* 2014). If this proves to be the case, it could in part explain the success of these plants as weed species in New Zealand. Also, any inoculum used on *Lupinus polyphyllus* is likely to nodulate gorse, European broom, tree lupin and tagasaste.

Conclusions

A diverse range of rhizobia genera and species nodulate legumes in New Zealand soils. It seems likely that further rhizobia will be associated with the many naturalised legumes still to be examined. Generally, native, crop and weed legumes are nodulated by different groups of rhizobia with crop legumes primarily effectively nodulated by rhizobia used as inoculum. However, lucerne can be nodulated by *Rhizobium* strains which give poor growth. It is not known how widespread these strains are and this warrants further study. Also, further work is required to assess the level of persistence of lucerne rhizobial inoculants in different soils in New Zealand. *Bradyrhizobia* that cross-nodulate lupins, gorse, European broom and tagasaste are of widespread occurrence in New Zealand and these require further characterisation. Also, further work is required to fully assess if *Lupinus polyphyllus* benefits from rhizobial inoculants in high country soils in the South Island..

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